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Comparative Effectiveness of Five Newly Synthesized Arsenical Compounds in Treatment of Leukemia in Mice. (19666)

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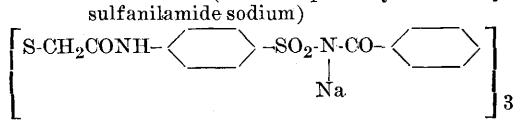
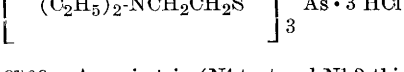
Arsenic in the form of potassium arsenite (Fowler's solution) is an effective therapeutic agent in chronic myelogenous leukemia. Theoretically the arsenic acts as a general poison by combining with the thiol compounds present in protoplasm, probably SH components of the pyruvate oxidase enzyme system(1). In chronic myelogenous leukemia there seems to be a selective action on the myeloid cells(2). It is our impression that this selective action of Fowler's solution on myeloid elements is greater than that of other agents such as urethane and irradiation so that fewer instances of concurrent depression of the other marrow elements occur. It therefore seemed desirable to study the effects of other arsenical compounds in an effort to find one of greater therapeutic efficiency. In this study 5 newly synthesized trivalent organic arsenical compounds (with the arsenic not attached to a benzene ring) were compared along with potassium arsenite for their ability to prolong the lives of mice affected with transmitted leukemia. In planning the types of organic arsenic compounds to be tested, it seemed desirable to so modify the organic "carrier radical" that transport across cell membranes might significantly differ with each type. The identifying MC numbers, structures and names of the compounds tested are shown below:

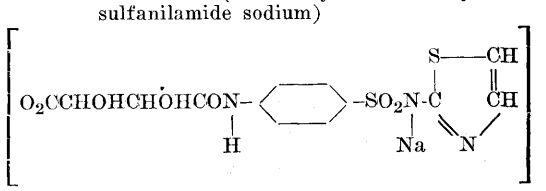
MC 2186—Arsenic-tris-(N^4-mercaptoacetyl-N^1-benzoyl sulfanilamide sodium)



MC 2767—Tris-(β-Diethylaminoethylthio)arsine hydrochloride

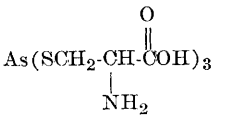


MC 2768—Arsenic-tris-(N^4-tartaryl-N^1-2-thiazolyl-sulfanilamide sodium)



MC 2864—Tris (β-hydroxyethylthio) arsine $(HOCH_2CH_2S)_3$ As

MC 2865—Tris (β-carboxy-β-aminoethylthio) arsine



In Type 1 (MC 2186 and 2865), arsenic is bound to sulfur as part of an anion; in Type 2 (MC 2767), arsenic is bound to sulfur as part of a cation; in Type 3 (MC 2864), arsenic is bound to sulfur in a neutral molecule;

TABLE I. Results of Toxicity Studies in Mice.

Drug	Approx. molecular wt	Arsenic atoms per molecule	Max tolerated dose, mg/kg	Dosage used in therapeutic exp., mg/kg	Relative amt of arsenic per therapeutic dose; potassium arsenite = 1
Potassium arsenite	146	1	15	7.5*	1
MC-2186	1522	1	80	80	1
2767	748	1	40	40	1
2768	1633	1	320	320	4
2864	306	1	10	10	1
2865	433	1	40	20-40†	1-2

* This dosage was used so that the studies could be roughly compared with similar studies done by Burchenal *et al.*(3).

† Four animals in exp. 2 received only 20 mg/kg.

and in Type 4 (MC 2768), arsenic is bound to oxygen as part of an anion. Thus, we were afforded an opportunity to gain some insight as to the chemical structural requirements for activity in the treatment of experimental leukemia.

Materials and methods. Mice of the Ak strain were used in all experiments. The toxicity studies were performed with mice of the Ak strain obtained from the Rockland Farms. All therapeutic studies, however, were performed using Ak_m mice inbred in our own laboratory. Practically all of this strain of mice die of leukemia following the intraperitoneal injection of leukemic cells from another mouse. The mice used in the experiments varied in age from 6 to 9 weeks. Leukemia does not occur spontaneously until later in life.

Tumors. Two transmissible tumors were used. 1. Lymphoid leukemia 1527. This developed spontaneously in a mouse of the Ak_m strain. A subcutaneous tumor of lymphoid tissue could be produced by injecting the cells subcutaneously. After intraperitoneal injection the animals all died of a generalized leukemia with a marked elevation of the white blood cell count and the presence of many lymphoblasts in the peripheral blood. There was a generalized lymph node enlargement and a moderate enlargement of the spleen and sometimes the liver. The tumor was observed through several transmissions before it was used so that its natural history could be studied.

2. Myeloid leukemia 1067. This also developed spontaneously in a mouse of the Ak_m strain. The mouse was called to our attention by the relatively large size of the spleen as compared to the lymph nodes. Blood smears from the original mouse as well as from mice to which the tumor was transmitted revealed a very high white blood cell count, many of the cells being immature and containing granules (promyelocytes). This impression was

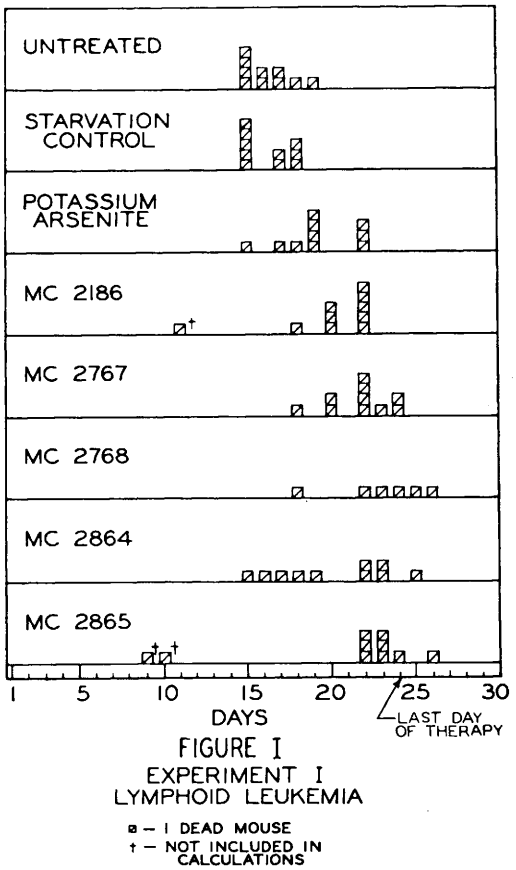


FIGURE I
EXPERIMENT I
LYMPHOID LEUKEMIA

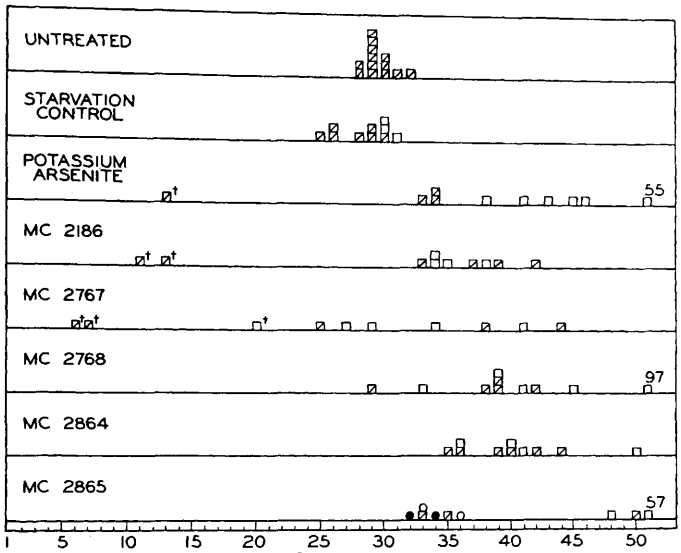


FIGURE II
EXPERIMENT 2
MYELOID LEUKEMIA

ø—●—1 DEAD MOUSE
CIRCULAR SYMBOLS MARK MICE THAT RECEIVED HALF THE USUAL DOSE OF THE DRUG
OPEN SYMBOLS MARK MICE THAT RECEIVED THERAPY BEYOND THE REGULAR 10 INJECTIONS
† — NOT INCLUDED IN CALCULATIONS

confirmed by staining the smears with peroxidase stains. The cells were not used in the experiments until they were transferred several times in order to be sure that no appreciable change occurred in the characteristics of the cells.

Compounds tested. All of the compounds were dissolved in physiological saline. In the case of MC 2865 it was necessary to add a few drops of NaOH to effect solution. The concentrations were adjusted so that the volume of injected material per mouse was in the range .04 to 0.32 ml in the toxicity experiments and 0.06 to 0.24 ml in the therapeutic experiments. Within any one group of animals the volume of injected material was almost constant.

The methods used were modifications of those described by Burchenal *et al.*(3). The maximum tolerated dose of the therapeutic agents for Ak mice was first determined. For each drug tested at least 15 mice were used, 3 mice for each of 5 dosages. Usually the doses selected were from the following: 10, 20, 40, 80, 160, 320, 640, and 960 mg/kg. In some cases intermediate doses were also tested. The mice were injected intraperitoneally daily for 7 days. The animals were weighed daily. Appreciable loss of weight and death were considered signs of toxicity. The drugs were then tested for their ability to prolong the lives of mice carrying transmitted leukemia. Eight groups consisting of 10 mice each were used in each of 2 experiments. The lymphoid leukemia tumor was used in the first experiment and the myeloid leukemia in the second. In each experiment the following groups were studied: untreated control, starvation control, Fowler's solution treated, and the 5 groups treated with the new arsenicals. The starvation control group were fed one g of bread wet with milk plus 0.75 g of chow daily per mouse. Water was given *ad lib.*(4). One million cells (0.1 cc) of a saline suspension of minced subcutaneous tumor in the first experiment and of minced spleen and lymph node in the second experiment were injected intraperitoneally into all of the mice. Forty-eight hours later the animals were divided into the 8 groups and therapy or the starvation diet was started. The drugs were injected intraperitoneally 3 times a week (MWF) for a total of 10 injections. In the second experiment a few of the animals of each group were

TABLE II.

Group	No. of mice	Survival time, days Range	Mean	Stand. error, Sm
	First exp.			
Combined untreated control	20	15-19	16.3	.307
Potassium arsenite	10	15-22	19.2*	.727
MC-2186	9	18-22	20.9*	.485
2767	10	18-24	21.7*	.597
2768	6	18-26	23 *	1.157
2864	10	15-25	20 *	1.087
2865	8	22-26	23.1*†	.481
	Second exp.			
Combined untreated control	23	25-32	29	.073
Potassium arsenite	9	33-55	41 *	.845
MC-2186	8	33-42	36.5*†	.410
2767	7	25-44	34 *†	1.127
2768	10	29-97	44.2*	2.010
2864	10	35-50	40.3*	.469
2865	9	32-57	39.8*	1.094

* These means are significantly different from the mean of the combined untreated control group on the 1% level (Fisher and Yates) (5).

† These means are significantly different from the mean of the potassium arsenite treated group on the 1% level.

continued on therapy beyond the 10 injections. These are so marked on the chart. The mice were weighed frequently. In a few cases the dosage of the drug was reduced if it was felt that the animal was developing drug toxicity. At death the animals were examined grossly for evidences of disease. The statistical significance of the results was calculated by using a method described by Fisher and Yates(5) for determining the significance between 2 Student distributions. The treated groups were compared with the combined untreated control group. The groups treated with the new arsenicals were compared to the potassium arsenite treated group.

Results. Table I shows the results of the toxicity studies. It will be seen that mice can tolerate about 4 times as much arsenic when given in the form of MC 2768 as compared to any of the other compounds.

Fig. 1 and 2 demonstrate the results of the 2 therapeutic experiments. In the first experiment (lymphoid leukemia) the 3 mice that died before the 12th day were losing weight and probably died from drug toxicity. These were not included in the significant statistics. Since there was no appreciable difference between the untreated control group and the starvation control group, these together were considered the combined untreated control group. As can be seen in Table II, there is a significant difference between each of the treated groups and the combined untreated control group. When each of the groups treated with the new arsenical compounds is compared to the group treated with potassium arsenite, only the group treated with MC 2865 shows a difference which is statistically significant (probability $<.01$).

In the second experiment (myeloid leukemia) 6 animals which died before the 21st day were not included in the significance calculations since they showed signs of severe drug toxicity. The untreated control group and the starvation control group were considered as one combined untreated control group as in the first experiment. All of the treated groups are significantly different from the combined untreated control group (Table II). Two of the groups treated with the new arsenical compounds (MC 2186 and MC 2767) are of significantly less value when compared to the group treated with potassium arsenite. None of the drugs is significantly better than the potassium arsenite.

Summary. While all of the treated groups of animals showed a statistically significant difference from the untreated control groups, there was very little difference between the treated groups. In one group, MC 2865 in Exp. 1, the new drug was probably more efficacious than potassium arsenite in the dosages used.

The technical assistance of Virginia Havel is gratefully acknowledged.

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